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(54) Nanbv diagnostics and vaccines.

(57) A family of cDNA sequences derived from hepatitis C virus (HCV) are provided. These sequences encode antigens which react immunologically with antibodies present in individuals with non-A non-B hepatitis (NANBH), but which generally are absent from individuals infected with hepatitis A virus (HAV) or hepatitis B virus (HBV), and also are absent from control individuals. A comparison of these cDNA sequences with the sequences in Genebank, and with the sequences of hepatitis delta virus (HDV) and HBV shows a lack of substantial homology. A comparison of the sequences of amino acids encoded in the cDNA with the sequences of Flaviviruses indicates that HCV is a Flavivirus or Flavi-like virus.

The HCV cDNA sequences are useful for the design of polynucleotide probes, and for the synthesis of polypeptides which may be used in immunoassays. Both the polynucleotide probes and the polypeptides may be useful for the diagnosis of HCV-induced NANBH, and for screening blood bank specimens and donors for HCV infection. In addition, these cDNA sequences may be useful for the synthesis of immunogenic polypeptides which may be used in vaccines for the treatment, prophylactic and/or therapeutic, of HCV infection. Polypeptides encoded within the cDNA sequences may also be used to raise antibodies against HCV antigens, and for the purification of antibodies directed against HCV antigens. These antibodies may be useful in immunoassays for detecting HCV antigens associated with NANBH in individuals, and in blood bank donations. Moreover, these antibodies may be used for treatment of NANBH in individuals.

The reagents provided in the invention also enable the isolation of NANBH agent(s), and the propagation of

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these agent(s) in tissue culture systems. Moreover, they provide reagents which are useful for screening for antiviral agents for HCV, particularly in tissue culture or animal model systems.

from clones 81, 40b, and 25c. This mixture was used to increase the sensitivity of the hybridization assay. The samples in panel I were hybridized with the plus strand probe mixture. The samples in panel II were probed by hybridization with the minus strand probe mixture. The composition of the samples in the panels of the immunoblot are presented in table 4.

Table 4

lane	A	B
1	HCV genome	*
2	----	*
3	*	cDNA 81
4	----	cDNA 81

* is an undescribed sample.

As seen from the results in Fig. 41, only the minus strand DNA probe hybridizes with the isolated HCV genome. This result, in combination with the result showing that the genome is sensitive to RNase and not DNase (See Section IV.C.2.), suggests that the genome of NANBV is positive stranded RNA.

These data, and data from other laboratories concerning the physicochemical properties of a putative NANBV(s), are consistent with the possibility that HCV is a member of the Flaviviridae. However, the possibility that HCV represents a new class of viral agent has not been eliminated.

IV.H.2. Detection of Sequences in Captured Particles Which When Amplified by PCR Hybridize to HCV cDNA Derived from Clone 81

The RNA in captured particles was obtained as described in Section IV.H.1. The analysis for sequences which hybridize to the HCV cDNA derived from clone 81 was carried out utilizing the PCR amplification procedure, as described in Section IV.C.3, except that the hybridization probe was a kinased oligonucleotide derived from the clone 81 cDNA sequence. The results showed that the amplified sequences hybridized with the clone 81 derived HCV cDNA probe.

IV.H.3. Homology Between the Non-Structural Protein of Dengue Flavivirus (MNWWVD1) and the HCV Polypeptides Encoded by the Combined ORF of Clones 14i Through 39c

The combined HCV cDNAs of clones 14i through 39c contain one continuous ORF, as shown in Fig. 26. The polypeptide encoded therein was analyzed for sequence homology with the region of the non-structural polypeptide(s) in Dengue flavivirus (MNWVD1). The analysis used the Dayhoff protein data base, and was performed on a computer. The results are shown in Fig. 42, where the symbol (:) indicates an exact homology, and the symbol (.) indicates a conservative replacement in the sequence; the dashes indicate spaces inserted into the sequence to achieve the greatest homologies. As seen from the figure, there is significant homology between the sequence encoded in the HCV cDNA, and the non-structural polypeptide-(s) of Dengue virus. In addition to the homology shown in Fig. 42, analysis of the polypeptide segment encoded in a region towards the 3'-end of the cDNA also contained sequences which are homologous to sequences in the Dengue polymerase. Of consequence is the finding that the canonical Gly-Asp-Asp (GDD) sequence thought to be essential for RNA-dependent RNA polymerases is contained in the polypeptide encoded in HCV cDNA, in a location which is consistent with that in Dengue 2 virus. (Data not shown.)

IV.H.4. HCV-DNA is Not Detectable in NANBH Infected Tissue

Two types of studies provide results suggesting that HCV-DNA is not detectable in tissue from an individual with NANBH. These results, in conjunction with those described in IV.C. and IV.H.1. and IV.H.2. provide evidence that HCV is not a DNA containing virus, and that its replication does not involve cDNA.

IV.H.4.a. Southern Blotting Procedure

FIG. 41-1

Homology between the HCV polypeptide encoded by combined ORF of clones 14i through 39c) and the non-structural protein of the Dengue flavi-virus(MNWVD1).

```
                10        20        30        40        50
HCV      EYVVLLFLLLADARVCSCLWMMLLISQAEAALENLVILNAASLAGTHGLVSFLVFFCFA

MNWVD1   AVSFVTLITGNMSFRDLGRVMVMVGATMTDDIGMGVTYLALLAAFKVRPTFAAGLLLRKL
             130       140       150       160       170       180

        60        70        80        90       100       110
HCV      WYLKGKWVPGAVYTFYGMWPLLLLLLALPQRAYALDTEVAASCGGVVLVGLMALTLSPYY

MNWVD1   TSKELMMTTIGIVLLSQSTIPETILELTDALALGMMVLKMVRKMEKYQLAVTIMAILCVP
             190       200       210       220       230       240

       120       130       140       150       160       170
HCV      KRYISWCLWWLQYFLTRVEAQLHVWIPPLNVRGGRDAVILLMCAVHPTLVFDITKLLLAV
                    :   .  .  :  .  ......  .....  ..  :  ....:: .:  .:.:  .
MNWVD1   NAVILQNAWKVSCTILAVVSVSPLFLTSSQQKADWIPLALTIKGLNPTAIF-LTTLSRTN
             250       260       270       280       290

       180       190       200       210       220       230
HCV      FGPLWILQASLLKVPYF-VRVQGLLRF-CALARKMIGGHYVQMVIIKLGALTGTYVYNHL
          . : :..... :     . . .::.   ...  ...:  .  .  .  :..  ...   .
MNWVD1   KKRSWPLNEAIMAVGMVSILASSLLKNDIPMTGPLVAGGLLTVCYV-LTGRSADLELERA
       300       310       320       330       340       350

        240       250       260       270       280       290
HCV      TPLRDWAHNGLRDLAVAVEPVVFSQMETKLITWGADTAACGDIINGLPVSARRGREILLG
         . .. :...  . .. ..  ....:.  ....  . ......   .:.    .  . . :  :.
MNWVD1   ADVK-WEDQAEISGSSPILSITISE-DGSMSIKNEEEEQTLTILIRTGLLVISG---LFP
        360       370       380       390       400       410

        300       310       320       330       340       350
HCV      PADGMVSKGWRLLAPITAYAQQTRGLLGCIITSLTGRDKNQVEGEVQIVSTAAQTFLATC
         .  ...  ..:.   . .  :  .  ...   .. .. ....   .  .  . . .:.  ...
MNWVD1   VSIPITAAAWYLWEVKKQRAGVLWDVPSPPPVGKAELEDGAYRIKQKGILGYSQIGAGVY
          420       430       440       450       460       470

        360       370       380       390       400       410
HCV      INGVCWTVYHGAGTRTIASPKGPVIQMYTNVDQDLV-----GWPAPQGSRSLTPCTCGSSD
          .:.  :..:  .  .  .        ..  .  ...:..:::    ::    .  ...  .
MNWVD1   KEGTFHTMWHVTRGAVLMHKGKRIEPSWADVKKDLVSCGGGWKLEGEWKEGEEVQVLALE
          480       490       500       510       520       530

         420       430       440       450       460       470
HCV      LYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGSSGGPLLCPAGHAVGIFRAAVCTRGV
          .:  ...  :   ...   ..  ...  ....  :.::.:..  :..::..  ..:  ::.
MNWVD1   PGKNPRAVQTKPGLFKTN--AGTIGAVSLDFSPGTSGSPIIDKKGKVVGLYGNGVVTRSG
          540       550        560       570       580       590

         480       490       500       510       520         530
HCV      AKAVDFIPVENLETTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKS--TKVPAAYAAQ
         : .  .  ..:.  ..  .: ..:.        ...  .  .::.  .:..:.
MNWVD1   AYVSAIAQTEK--SIEDNPEIEDDIFRK---RKLTIMDLHPGAGKTKRYLPAIVRGAIKR
           600        610          620       630       640

          540       550       560       570       580
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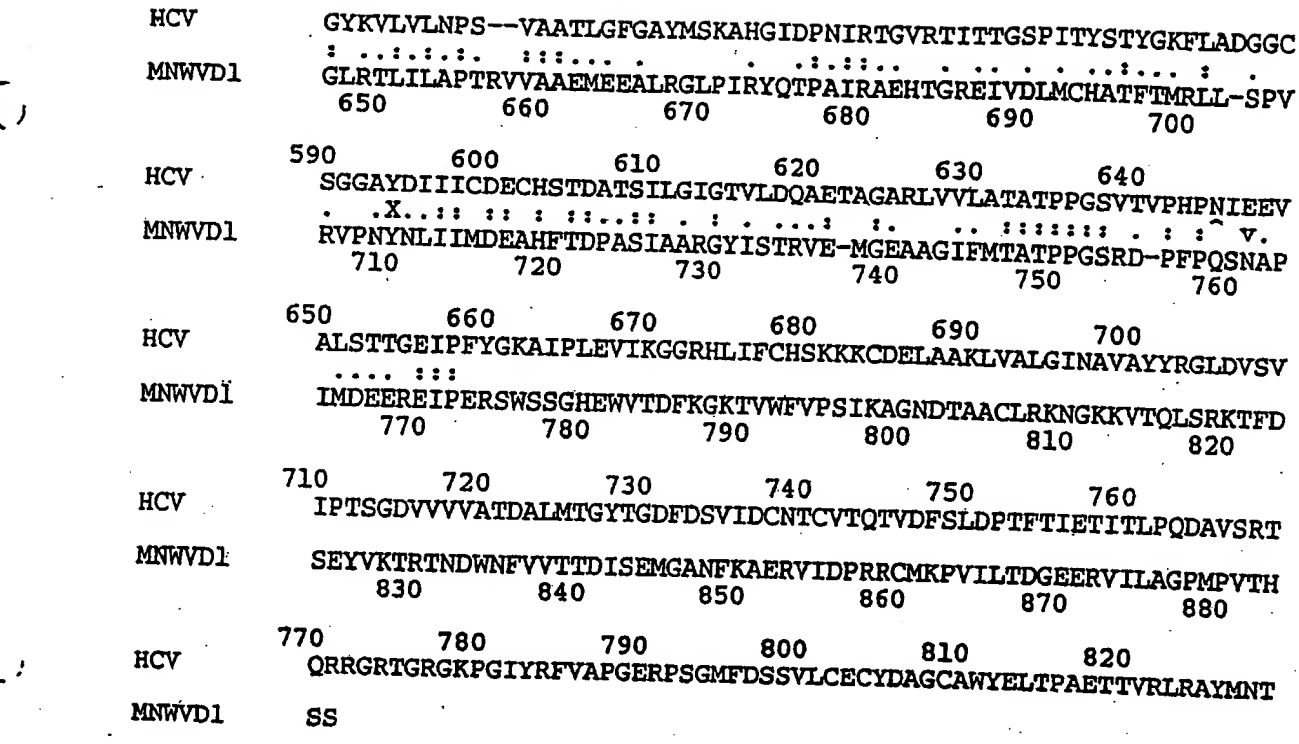
HCV GYKVLVLNPS--VAATLGFGAYMSKAHGIDPNIRTGVRTITTGSPITYSTYGKFLADGGC
MNWVD1 GLRTLILAPTRVVAAEMEEALRGLPIRYQTPAIRAEHTGREIVDLMCHATFTMRLL-SPV
650 660 670 680 690 700
590 600 610 620 630 640
HCV SGGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEV
MNWVD1 RVPNYNLIIMDEAHFTDPASIAARGYISTRVE-MGEAAGIFMTATPPGSRD-PFPQSNAP
710 720 730 740 750 760
650 660 670 680 690 700
HCV ALSTTGEIPFYGKAIPLEVIKGGRHLIFCHSKKKCDELAAKLVALGINAVAYYRGLDVSV
MNWVD1 IMDEEREIPERSWSSGHEWVTDFKGKTVWFVPSIKAGNDTAACLRKNGKKVTQLSRKTFD
770 780 790 800 810 820
710 720 730 740 750 760
HCV IPTSGDVVVVATDALMTGYTGDFDSVIDCNTCVTQTVDFSLDPTFTIETITLPQDAVSRT
MNWVD1 SEYVKTRTNDWNFVVTTDISEMGANFKAERVIDPRRCMKPVILTDGEERVILAGPMPVTH
830 840 850 860 870 880
770 780 790 800 810 820
HCV QRRGRTGRGKPGIYRFVAPGERPSGMFDSSVLCECYDAGCAWYELTPAETTVRLRAYMNT
MNWVD1 SS

FIG. 41-2